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THE EFFECT OF VARIOUS NUTRIENT SUPPLIES ON THE LEAF MORPHOLOGY AND ANATOMY OF THE *DIGITALIS LANATA*

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Abstract

The effect of the various doses of three basic nutrient elements (nitrogen, phosphorus, potassium) on the morphological and anatomical features of the *Digitalis lanata* rosette was studied under controlled condition. It could be determined that the leaf-number and-area change to a variable extent depending on the nutrient type, but in all cases following an optimum curve. On the contrary, the connection between the leaf thickness and the nutrient element levels could be regarded to be close to linear. Consequently the decrease of the leaf-number and-area experienced in the case of high doses is accompanied by the further increase of the leaf thickness.

The determination coefficients indicate that from the basic processes producing the morphological and anatomical changes, the differentiation and cell division — being elemental in the development of the leaf-number and-area — are affected by the phosphorus doses besides that of nitrogen. The cell enlargement, which is fundamental from the viewpoint of the leaf thickness, is modified mainly by the potassium amount.

Key words: *Digitalis lanata*, leaf morphology, leaf anatomy, nutrients.

Introduction

The response-reactions of plants to environmental effects are also expressed in the changes of their growth processes, which results in modification in the size, shape and mass ratio of the organs as well (WATSON and CASPER, 1984). From the viewpoint of morphology and anatomy, the most changable organ is the leaf (FAHN, 1982) which -in respect to the significance of photosynthesis- has firstly been studied with light, as an ecological factor. As general effect, among others the development of larger surfaced, thinner leaves (BJÖRKMAN, 1981) as well as the appearance of undeveloped palisade parenchyma (JACKSON, 1967) and large size intercellulars (FEKETE et al. 1973) are characteristic of the weak light.

At the same time, intensive illumination accelerates the formation of the palisade parenchyma and causes a decrease in the leaf area (SHIELDS, 1950). In many cases this is accompanied by the decrease in size of mesophyll cells too, resulting the increase of the internal surface (NOBEL, 1977). Among the effects of nutrients — first of all nitrogen phosphorus and potassium- the most well known are those of morphological nature, which have been described in detail in the work of BERGMAN

(1979). It is striking that there is relatively few data in respect to the anatomical changes concerning nutrient supply, which has comprehensively been studied both from physiological and production-biological points of view. Accordingly the characteristic of nitrogen-shortage is the inhibition in growth of the shoot, the formation of chlorotic and xeromorph leaves. In case of phosphorus shortage the symptoms are similar, but of slighter degree. In case of potassium shortage the small sized leaves also show characteristic „wilting" symptoms, caused by the shrinkage of the mesophyll cells. At high nitrogen level, besides the intensive growth, there is an increase in the ratio of the parenchyma cells contrary to the supporting elements, which makes the leaves „loose and soft".

In the case when the leaf signifies the utilizable organ, the modification of the leaf structure by the effect of nutrients is of particular importance. The heart-glycosides used as a basic material for medicaments occur in highest amounts in the leaf parenchyma of the *Digitalis lanata* (VOGEL and LUCKNER, 1981), thus the leaf size and mesophyll structure modified by nutrient supply may play important role in the development of the drug production and glycosid quantity. In connection with the above mentioned factors, but mainly in respect to production, a lot of contradictory data are known, which are summarized by BAALBA et al. (1971). Both stimulation, and inhibition of growth on the effect of nutrients are discussed in the cited paper, which can presumably be led back to the interaction of several climatic factors, or to the different nutrient element ratio.

In the present paper attempt is made to clarify the effect of three basic nutrient elements (nitrogen phosphorus, potassium) and their various doses on the leaf-number and-area, as well as anatomy of the *Digitalis lanata*, under conditioned condition. With this, authors wish to improve the knowledge on the morphological-anatomical background of the increase in glycoside production.

Material and method

The studies were performed in PGB-36(Conviron) type phytotron chamber using *Digitalis lanata* EHRH cv. „Oxfordi" plants. During the growth period the strenght of the illumination was 16 klx, and the phase 14 hours. The light energy was served by light tubes in 86% (F72 T12 (CW/Who) and by incandescent lamps in 14%. The relative humidity in the light/dark periods was 60/70%. In accordance with the values characteristic to the certain sections of the average 169 days long natural vegetation period, the temperature was as follows:

period	day(°C)	night(°C)
1– 2 weeks	16	10
3– 7 "	18	12
8–11 "	20	12
12– "	21	12

1-1 plant was grown in the culture dishes of 154 cm² area and 1300 cm³ volume, in the equal massproportioned mixture of sand-perlite, using modified KNOP nutrient solution. The nitrogen, phosphorus and potassium contents of the nutrient solution differed in each variant (Table 1.). The effect of the quantitative changes in the various nutrient elements was examined besides the median dose and

Table 1: Nutrient solutions dosed per plant in 10 day-period

Nutrient level	N dose(mg)	P dose(mg)	K dose(mg)
$N_1P_3K_3$	5.0	50.0	50.0
$N_2P_3K_3$	25.0	50.0	50.0
$N_3P_3K_3$	100.0	50.0	50.0
$N_4P_3K_3$	200.0	50.0	50.0
$N_5P_3K_3$	400.0	50.0	50.0
$N_3P_1K_3$	100.0	2.5	50.0
$N_3P_2K_3$	100.0	12.5	50.0
$N_3P_4K_3$	100.0	100.0	50.0
$N_3P_5K_3$	100.0	200.0	50.0
$N_3P_3K_1$	100.0	50.0	2.5
$N_3P_3K_2$	100.0	50.0	2.5
$N_3P_3K_4$	100.0	50.0	100.0
$N_3P_3K_5$	100.0	50.0	200.0

The nutrients were applied in the form of NH_4NO_3
NaH_2PO_4 and
KCl

unchanged ratio respectively, of the other two. 9-9 plants were evaluated per variant, processed on the 169th day following sowing in conformity with the average lenght of the natural vegetation period. During the course of the evaluation the leaf number, and leaf area were determinated. 50-50 measurements per variant were accomplished for the analysis of the mesophyllum structure. The sections were prepared according to the description given in our previous paper (MIHALIK and BERNÁTH, 1984). The determination coefficient were defined by the method of SVÁB (1973).

Results and discussion

1. RELATIONSHIP BETWEEN THE LEAF NUMBER PER PLANT AND THE NUTRIENT SUPPLY

The leaf number (Table 2.) is influenced to greatest extent by the nitrogen from the studied nutrient elements. With nitrogen insufficiency (N_1 variant) there is a considerable slackening in growth and fewer are formed by more than 60% compared to the formations at medium nutrient level (N_3). Higher nitrogen doses also result a significant decrease in the leaf number, moreover, even symptoms of intoxication can be detected in the case of the N_5 variant: The initial fast growth is followed by chlorosis, then by destruction of the leaves. Even if phosphorus amount is increased, the optimum of the leaf number is experienced at the medium level (P_3). The shortage of phosphorus is only of slight degree, while the excess brings forth a considerable decrease, however preceivable toxic symptoms do not appear in this case. Among the studied nutrients the effects of potassium is the slightest, disregarding extreme potassium insufficiency the leaf number is practically constant.

Table 2.: Leaf number and leaf area per plant

Nutrient level	leaf no.	leaf area(cm^2)
$N_1P_3K_3$	53.3	3400
$N_2P_3K_3$	97.5	4812
$N_3P_3K_3$	124.7	7821
$N_4P_3K_3$	99.6	4756
$N_3P_1K_3$	113.1	5343
$N_3P_2K_3$	124.2	5887
$N_3P_4K_3$	116.8	5322
$N_3P_5K_3$	79.1	3527
$N_3P_3K_1$	101.2	5689
$N_3P_3K_2$	128.2	5656
$N_3P_3K_4$	121.0	6110
$N_3P_3K_5$	125.3	5590

2. CHANGES IN LEAF AREA

The leaf area is formed along a curve optimal with the quantitative increase of all three nutrient elements. The maximum of the curve is observable at the $N_3P_3K_3$ level. As the result of the growth inhibition caused by the nitrogen insufficiency and phosphorus redundancy, the leaf area is the smallest in the case of N_1 and P_5 doses. The potassium has no basic importance here either, since the difference between the variants is relatively slight.

3. THE THICKNESS AND STRUCTURE OF THE LEAF

In contrast to the characteristics studied hitherto, the leaf thickness shows a close to linear increase with the rise of the nutrient level (Table 3). The linearity is the best evident in the case of nitrogen, it shows a transition towards the asymptotic curve in the case of phosphorus, while it only prevails with tendency character upon the increase of the potassium level. At low nitrogen and phosphorus levels it is not only less and of smaller size, but thinner leaves are formed as well. The effect of the potassium insufficiency is not unambiguous, however, its redundancy among the studied nutrients levels results in the formation of the thickest leaves. The leaf thickness firstly depends on the lenght of the palisade cells, and to lesser extent on the number of cell rows (Table 3.), the thickness of the spongy parenchyma is practically constant under our experimental conditions. In the case of nitrogen and phosphorus insufficiency the columnar parenchyma cells are cuboid, the „palisade character" is not expressed (Plate 1, fig. 1.). This phenomenon cannot be experienced in the case of potassium insufficiency. On the effect of high doses the thickness of

Table 3: Anatomic characteristic

Nutrient level	leaf thickness (μm)	palisade parenchyma thickness (μm)	cell rows no.
$N_1P_3K_3$	247.8	79.2	2.5
$N_2P_3K_3$	260.4	93.9	2.0
$N_3P_3K_3$	296.8	99.9	2.4
$N_4P_3K_3$	339.8	125.2	2.7
$N_3P_1K_3$	232.1	78.6	2.0
$N_3P_2K_3$	299.0	111.8	2.8
$N_3P_4K_3$	313.7	120.7	2.8
$N_3P_5K_3$	314.5	121.8	2.7
$N_3P_3K_1$	317.0	124.9	2.8
$N_3P_3K_2$	299.8	113.5	2.8
$N_3P_3K_4$	364.8	141.1	2.9
$N_3P_3K_5$	348.9	160.8	3.0

the palisade layer suprasses the value of the moderate nutrient element level by 20-50%, and the increase is the highest in the case of the K_4 variant (Plate 1. fig. 3.).

Apart from the mesophyll structure, the quality of the cell walls is also changed by the nutrient supply. In the case of nitrogen insufficiency the cell walls are thin and only weakly stainable; on the effect of phosphorus redundancy they become stiff and fragile, thus the leaves can only be cut with difficulty.

4. CHARACTERIZATION OF THE EFFECTIVENESS OF THE NUTRIENT ELEMENTS

The determination coefficient may provide us with information regarding the degree to which the nutrients and their combinations contribute at the development of the various changes (Table 4.). The role of the nutrient eleme is relatively moderate in the development of the leaf number. The effects of potassium seems to be negligible,

Table 4: Percentage values of determination coefficient

Nutrient elements	leaf number	leaf area	leaf thickness	palisade parenchyma thickness
N	19	33	32	23
P	15	29	8	5
K	4	14	18	35
N+P+K	38	76	58	63

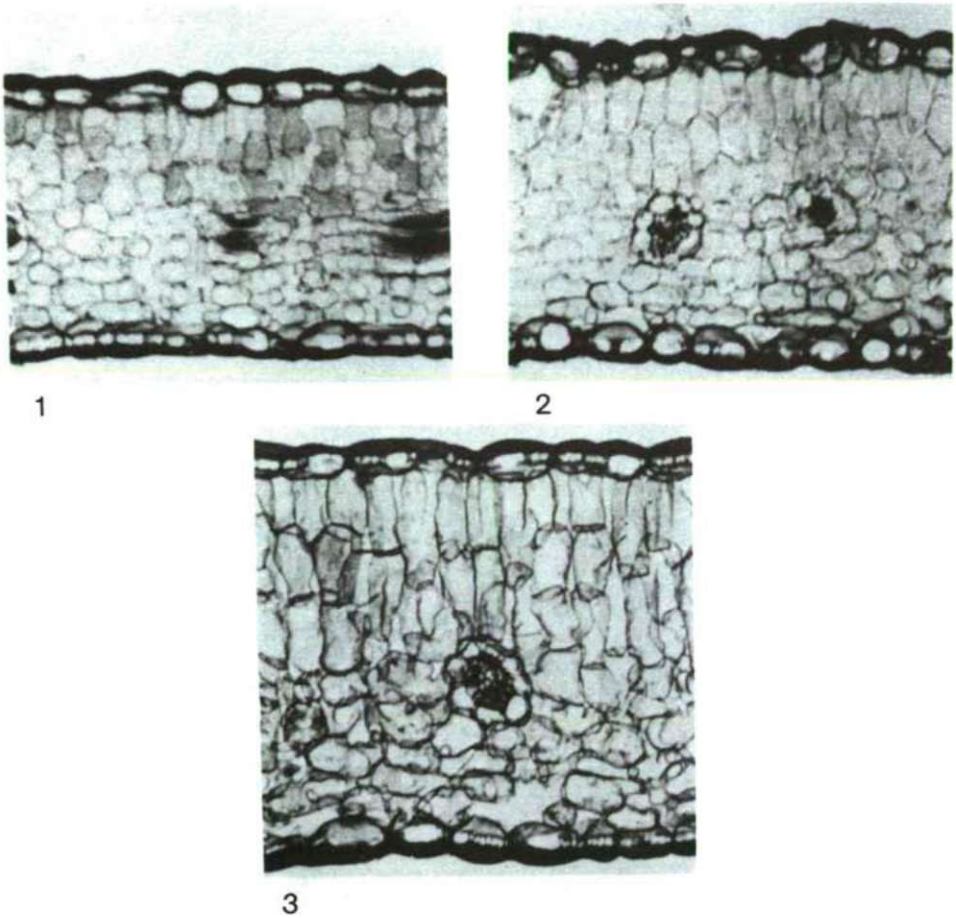

Plate 1. Tissue structure of the leaf mesophyll of *Digitalis lanata*: 1 = low nutrient level, 2 = medium nutrient supply, 3 = general effect of nutrient redundancy

only nitrogen and phosphorus influence the leaf formation slightly. The leaf area however depends to a greater extent on the nutrient element amount (76%). Similarly to the leaf number, apart from the nitrogen, the significance of phosphorus can be emphasized here, too, though an increase in the influence of potassium can be observed.

The leaf thickness as well as the thickness of the palisade layer determining this, respectively, also depend on the nutrient supply at the applied light intensity. It should be emphasized that contrary to previous two features, besides the nitrogen, the role of potassium becomes greater. This is particularly striking in the case of the palisade parenchyma thickness, where the determination coefficient of potassium is 35%.

According to our assumptions the influence of the nutrient elements goes through two different directions. In the development of the leaves and growth of the leaf area where the cell division dominates, the effects of nitrogen and phosphorus could be demonstrated successfully. In the cell enlargement, which is the main factor the growth in leaf thickness, the role of potassium is more significant.

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